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Alternative technologies for management of soil-borne diseases in bareroot forest nurseries in the United States

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ABSTRACT

Many forest nurseries producing bareroot seedlings in the United States rely on soil fumigation with methyl bromide and other chemicals to control soil-borne diseases. Methyl bromide production and importation will be phased out by the U.S. Environmental Protection Agency by the year 2000. Growers need alternative production technologies to grow high quality seedlings without fumigation because substitute fumigants are undesirable due to environmental and human health hazards. The Forest Pest Management branch of the USDA Forest Service recently instituted field trials at several U.S. nurseries to evaluate alternative cropping regimes and determine their effects on soil-borne diseases. Various organic amendments, fallow periods, cultivation regimes, and cover crops will be compared to methyl bromide fumigation. Additional studies will evaluate beneficial microorganisms and suppressive soils, develop accurate sampling techniques for differentiating pathogenic *F. oxysporum* populations, karyotype *Fusarium* isolates, and develop ELISA assays for detection and quantification of plant pathogenic *Cylindrocladium* and *Macrophomina* spp.

INTRODUCTION

Most U.S. bareroot nurseries rely on chemical fumigation to reduce levels of soil-borne pathogenic fungi, nematodes, weeds, and insects. Soils are routinely fumigated every 2-3 years at approximately 90% of U.S. bareroot nurseries

Table 1. Non-methyl bromide chemical fumigants for use in forest tree nurseries and their characteristic target organisms (from Andersen and Lee-Bapty 1992).

Fumigant Formulation	Trade Name	Target Organisms
dazomet	*BasamidR*	Soil-borne pathogens, weeds, and insects
metam-sodium	*VapamR*	Soil-borne pathogens, nematodes,
	BusanR	weeds, and insects
77.9% 1,3 dicholo- propene	Telone *C-17R*	Soil-borne pathogens and nematodes
16.5% chloropicrin		
1,3 dichloropropene	Telone *IIR*	Soil-borne nematodes
20% methyl iso- thiocyanate	*VorlexR*	Soil-borne pathogens, weeds, insects and nematodes
40% dichloropropenes and dichloropropanes		
10% inert		
Chloropicrin	none	Soil-borne pathogens and nematodes

(Fraedrich, 1993). In the northern and western portions of the United States, 80% of the nurseries fumigate soil, whereas fumigation occurs at 96% of the southern nurseries. Many nurseries fumigate soil before each seedling crop, except in the southern United States where soils are often fumigated before every other third crop. However, each seedling crop produced in the southern United States usually require only one year, whereas crops in the western and northern states takes 2-3 years.

Methyl bromide, applied with chloropicrin, is used by almost all nurseries that fumigate their soil (Fraedrich, 1993; James, 1989). Although other chemical fumigants are available (table 1), most growers believe existing alternatives are not as effective as methyl bromide formulations. Dazomet (Basamid^{R}) fumigant has been tested at half of the nurseries that fumigate their soil, but results show it is often less effective than methyl bromide (Fraedrich, 1993).

Methyl bromide is widely used as a soil fumigant to produce strawberries, peppers, tomatoes, tobacco, citrus and ornamentals (NAPIAP, 1993). It is also used for post harvest treatment of fruits and vegetables, quarantine treatments, and structural fumigation. The 1991 Montreal Protocol Assessment alleged that methyl bromide was in a category of chemicals that depletes stratospheric ozone (NAPIAP, 1993). In response to this assessment, the United States

Environmental Protection Agency (EPA) under the Clean Air Act, called for phase out of production and importation of methyl bromide by the year 2000. Although there are many uncertainties regarding methyl bromide's relationship to ozone depletion, the phaseout is progressing. Annual monetary losses resulting from a total ban of methyl bromide use in U.S. agriculture are estimated at $1.3 to 1.5 billion (NAPIAP, 1993). The share of these losses attributed to forest tree nurseries amounts to $35 million/year.

Many nursery growers desire alternatives to chemical fumigation because of high costs of fumigation, health risks to nursery workers, visitors, neighbours and wildlife, and a desire to reduce environmental contamination, especially ground water supplies. Growers want an integrated pest management (IPM) approach for dealing with soil-borne diseases, weeds, and insect pests (Campbell, 1991; James *et al.*, 1990; Raupp and Cornell, 1988). Implicit in IPM is a formal decision-making process that considers pest populations and their impacts on hosts, and weighs the effectiveness of control methods against their impacts on economics, human health, and the environment. This approach requires much more information on biological interactions of soil microorganisms and a reliable system for disease prediction and monitoring of pathogen populations. Because fumigation with non-selective biocides kills all soil organisms, knowledge of the dynamics and interactions of soil micro-organisms have not been critical and little is currently known about them.

To prepare for the loss of methyl bromide, the Forest Pest Management branch of the U.S. Forest Service initiated alternatives to fumigation trials in four western states (California, Oregon, Washington, Idaho), Minnesota and Florida. This paper outlines the project's background, methods and expected outcomes.

RESEARCH BASIS

Although most U.S. forest nurseries routinely fumigate their soil, several western nurseries produce high-quality seedlings without fumigation. Methyl bromide fumigation is prohibited in Canada, so bareroot nurseries there have never fumigated. Yet they still produce high-quality seedlings. these precedents were examined to provide the basis for conversion from a reliance on fumigation to cultural and biological pest management methods.

Effects of *Brassica* cover crops, sawdust amendments, and fallowing with periodic cultivation on production of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings have been evaluated in several nurseries in Oregon and Washington (Stone, 1993). This research into alternative cropping regimes

indicates that quality conifer seedlings can be grown without soil fumigation by using bare fallow and cultivation. In this trial and others, cover crops stimulated increases in populations of potentially pathogenic organisms and may be detrimental to seedling health (Patrick and Toussoun, 1970; Stone 1993).

Research in agricultural systems shows that suppressive soils (those which may contain pathogens, but within which no disease occurs) may be induced by cultural manipulations including addition of organic amendments to soil (Toussoun, 1975; Sinclair *et al.*, 1975; Schisler and Linderman, 1989). In China, Japan, and other parts of the Orient, organic amendments have been used in agriculture with beneficial effects for many years (Hoitink and Fahy, 1986; Huang and Kuhlman, 1991; Kelman and Cook, 1977). Through cover crop incorporation, and/or organic soil amendment, microorganisms antagonistic toward or competitive with soil-borne pathogens may be stimulated, resulting in lower disease levels (Baker and Cook,, 1974; Ramirez-Villapudua and Munnecke, 1988). These soil treatments create a "biological balance" where pathogenic microorganism populations are kept low while beneficial competitors and antagonists are increased (Danielson and Davey, 1969; James, 1989; Vaartaja, 1967). It may take several cropping cycles to achieve the desired "biological balance" of microorganisms, but once established, such communities are quite stable and should remain so indefinitely unless extensive disturbances occur, such as fumigation (James, 1989).

Biological control has reduced soil-borne pathogens in agricultural systems and has great potential in forest tree nurseries (Baker and Cook, 1974; Campbell, 1989; Papavizas, 1985; Van Wyk *et al.*, 1988). Antagonistic and competitive fungi and bacteria provide control of many soil-borne pathogens (Baker and Cook, 1974; Elad and Baker, 1985; Sivan and Chet, 1989). Since commercial biocontrol agents have been developed for agricultural crops other than forest tree seedlings, these agents need to be critically evaluated for efficacy in forest nurseries and integrated with other practices designed to enhance soil suppressiveness. Screening of potential biocontrol agents obtained from forest nursery soils is also needed to determine if nursery specific or region-wide biocontrol agents may be found.

Improvements in monitoring and detecting soil-borne pathogens are needed. Using current assay procedures, *Fusarium* and *Pythium* population levels have not been useful for predicting seedling disease development; there is often little or no relationship between soil population levels of *Fusarium* and *Pythium* and levels of disease on seedlings. This is primarily due to our inability to distinguish between pathogenic and non-pathogenic strains of these fungi. Pathogenic and non-pathogenic strains can be morphologically similar (Bloomberg, 1966; Booth,

1971) so total populations are not accurate disease predictors. Recently refined molecular techniques may be valuable for differentiating among fungal genera, species, and strains (Kerr, 1987; Young, 1990). Pulsed-field gel electrophoresis, polymerase chain reaction, random primed amplified DNA, and vegetative compatibility groupings will be used to measure genetic differences of fungi (Gordon and Okamota, 1992; Puhalla, 1985). These techniques will be adapted for use in assays to quickly differentiate pathogenic and non-pathogenic fungal strains within soil and infected plant material without having to resort to burdensome pathogenicity tests.

This project will answer four major questions:

1). Can alternative cropping techniques bare fallow, cover crops, and soil amendments, be used to manage soil-borne diseases in high-volume production nurseries?

2). Can biocontrol techniques, including beneficial microorganisms and suppressive soils adequately control soil-borne diseases in bareroot forest nurseries?

3). Can recent developments in biotechnology be applied to genetically differentiate pathogenic and non-pathogenic populations of *F. oxysporum* Schlecht in forest nursery soils and on seedling roots?

4). Can immunoassay techniques be developed for detecting *Cylindrocladium* and *Macrophomina* spp. which are as effective as existing cultural or other techniques of detection?

METHODS

In 1993, eight nurseries set up alternatives to fumigation trials in the western United States: three in California, one in Washington, and two each in Oregon and Idaho. One Florida nursery was included; nurseries in Minnesota, North Carolina and South Carolina will be added in 1994. The project will span four years to allow for bareroot seedling production in the western and northern regions: one year for pre-sowing treatments, two years for crop production, and one year for post-lifting analyses. In the southern region crops are grown annually; therefore, several crop cycles are possible during this timeframe.

Field treatments tailored to each nursery are being tested. Locally available soil amendments, cover crops adapted to local growing conditions and available equipment will be used to create soil conditions that minimize damage from soil-borne organisms. Treatments include the following organic amendments: sawdust, sewage sludge, composed mushroom media, pine bark, yard waste, and pine needle mulches. Most nurseries will test bare fallowing with periodic cultivation because of its reported success in British Columbia and Oregon and Washington (Stone, 1993). Standard chemical soil fumigation will be included for comparison. Treatments will be compared for seedling establishment, disease incidence and severity, seedling growth, and production of shippable seedlings per unit area.

Plots were set up using a complete randomized block design with buffer areas between treatments. Operational nursery procedures for fertilization, irrigation, and weed control are being used. One conifer species of one seedlot will be sown into all treatments to reduce inter-seedlot variability.

Fusarium populations are being monitored and estimates of pathogenic species will be made for each treatment (proportion of the *Fusarium* population that consists of *F. oxysporum*). Soil samples will be processed by one laboratory to reduce variability in assay procedures. Several samples of forest soil will be evaluated for inherent suppressiveness to soil-borne pathogens through greenhouse screening followed by field testing.

Because of its wide geographic distribution and importance in forest nurseries (James *et al.*, 1990), *F. oxysporum* will be used for most of the detection protocols. Representative *Fusarium* isolates will be maintained and techniques developed to rapidly elucidate pathogenicity. Pathogenic strains and host range variabilities will be identified and evaluated using molecular techniques to determine the genetic component of pathogenicity. Vegetative compatibility grouping, pulsed-field gel electrophoresis, and polymerase chain reaction to amplify probes will be tested to determine their ability to distinguish pathogenic and non-pathogenic strains. Population genetics of *F. oxysporum* within selected nurseries will be elucidated and quick assay techniques developed for determining the proportion of pathogenic strains within a population.

For detection of *Cylindrocladium* and *Macrophomina* enzyme-linked immunosorbent assays (ELISA) will be developed. The development and testing of ELISA kits will occur in three phases. In the first phase, antibody titers for the immunoassay will be produced from rabbits inoculated with antigens and Freund's complete adjuvant. Three types of testing will be conducted in the second phase: cross-reactivity with other common root pathogens and soil saprophytes, comparison studies with existing detection techniques for

Cylindrocladium and *Macrophomina*, and determination of sensitivity of the immunoassays. In the final phase, ELISA detection kits will be field tested.

COOPERATORS

This project will include several cooperators from government, universities, and private research laboratories. A listing of cooperators is in table 2.

Table 2. Cooperators investigating alternative technologies for management of soil-borne diseases in bareroot forest nurseries in the United States.

USDA Forest Service	
Forest Pest Management	Region 1 (Northern Region) Region 6 (Pacific Northwest Region) Region 5 (Pacific Southwest Region) Region 8 (Southern Region) Northeast Area - State and Private Forestry
Research	North Central Forest Experiment Station Southeastern Forest Experiment Station
State Organizations	Florida Division of Forestry California Department of Forestry Oregon State Department of Forestry
Universities	Oregon State University University of British Columbia Clemson University
Private Laboratories	B.C. Research (Vancouver, British Columbia, Canada) PENINSU-LAB (Poulsbo, Washington)

EXPECTED PRODUCTS

At the conclusion of this project, the following will be available:

1). Customized recommendations of cultural and biological treatments for management of soil-borne diseases without chemical fumigation.

2). Protocols for differentiating pathogenic and non-pathogenic populations of *F. oxysporum* and ELISA detection kits for *Cylindrocladium* and *Macrophomina*.

3). Information on the genetic makeup of *Fusarium*, nursery soil suppressiveness, and soil ecosystem interactions.

CONCLUSIONS

The transition from a reliance on chemical soil fumigation to growth regimes without fumigation will be difficult. Most cultural and biological techniques may have limited effectiveness and growers may have to accept higher than usual losses or lower seedling quality. Eventually, pest-caused losses should decline as soils become more suppressive (Toussoun, 1975). Improved detection tools will aid in understanding soil ecosystems and allow pathologists to predict effects of cultural treatments on disease development, thereby enabling growers to minimize losses.

REFERENCES

ANDERSON S.O., LEE-BAPTY S., 1992. Technology and economic assessment of methyl bromide uses, alternatives and substitutes. Report prepared for the United Nations Environment Programme on behalf of the Contracting Parties to the *Montreal Protocol*. 24p.

BAKER K.F., COOK R.J., 1974. Biological control of plant pathogens. W.H. Freeman and Co., San Francisco, CA. 433p.

BLOOMBERG W.J., 1966. The occurrence of endophytic fungi in Douglas-fir seedlings and seeds. *Canadian Journal of Botany* 44:413-420.

BOOTH C., 1971. The genus *Fusarium*. The Commonwealth Mycological Institute, Kew, Surrey, England. 237p.

CAMPBELL R., 1989. Biological control of microbial plant pathogens. Cambridge University Press, Cambridge, England. 218p.

PUHALLA J.E., 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Canadian Journal of Botany* 63:179-183.

RAMIREZ-VILLAPUDUA J., MUNNECKE D.E., 1988. Effect of solar heating and soil amendments of cruciferous residues on *Fusarium oxysporum* f. sp. *conglutinans* and other organisms. *Phytopathology* 78:289-295.

RAUPP M.J., CORNELL C.F., 1988. Pest prevention: treating pests to the IPM method. *American Nurseryman* 167(3):59-67.

SCHISLER D.A., LINDERMAN R.G., 1989. Influence of humic-rich organic amendments to coniferous nursery soils on Douglas-fir growth, damping-off and associated soil microorganisms. *Soil Biology and Biochemistry* 21:403-408.

SINCLAIR W.A., COWLES D.P., HEE S.M., 1975. Fusarium root rot of Douglas-fir seedlings: suppression by soil fumigation, fertility management, and inoculum with spores of the fungal symbiont *Laccaria laccata*. *Forest Science* 21:390-399.

SIVAN A., CHET I., 1989. The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. *Phytopathology* 79:198-203.

STONE J., 1993. Alternatives to chemical fumigants for the control of Fusarium root disease in forest nurseries - final report. Report of the Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR. 42p.

TOUSSOUN T.A., 1975. Fusarium suppressive soils. Pages 145-151 in G.W. Bruell, editor. Biological control of soil-borne plant pathogens. The American Phytopathological Society, St. Paul, MN.

VAARTAJA O., 1967. Reinfestation of sterilized nursery seedbeds by fungi. *Canadian Journal of Microbiology* 13:771-776.

VAN WYK P.S., SCHOLTZ D.J., MARASAS W.F.O., 1988. Protection of maize seedlings by *Fusarium moniliforme* against infection by *Fusarium graminearum* in the soil. *Plant and Soil* 107:251-257.

YOUNG N.D., 1990. Potential applications of map-based cloning to plant pathology. *Physiological and Molecular Plant Pathology* 37:81-94.

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ABSTRACT

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